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Research Article

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Effect of *Matricaria chamomilla* L Extract in Comparison with Ampicillin on Reproductive Histological Indices and Rate of Fertility in Male Mice Treated with *E. Coli* Lipopolysaccharide

Akram Hassan Zahraei[1], Mehrdad Modaresi[2]*

[1]Department of Biology, Payam e Noor University, Isfahan Center, Isfahan, Iran
[2]Department of Physiology, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran

Abstract The aim of this study was to evaluate the effects of chamomile (*Matricaria chamomilla L*) and extract in comparison with ampicillin on the histological parameters of the reproductive system in male mice treated with *E. coli* LPS. In this research, 65 adult male mice were divided into 6 groups. Control, LPS, LPS+ampicillin, LPS+chamomile extract 50 mg/kg, LPS+chamomile extract 100 mg/kg and LPS+chamomile extract 200 mg/kg. IP injection were performed for 20 days and the fertility of each group was evaluated. The results of histological and fertility tests of each group were compared with the control group. The results of histological studies showed a significant decrease in mean number of stem cells and primary spermatocyte cells of LPS group compared to that in the control group ($p \leq 0.05$). The diameter of the seminiferous tubules and the number of embryos in the five treatment groups show a significant change compared to control group ($p > 0.05$). According to these results, LPS can reduce stem cells and primary spermatocyte in the testicles and thereby reduce the fertility potential, while the treatment of chamomile extract in all three doses of 50, 100 and 200 can reduce the effects of LPS and increase fertility potential.

Keyword: Chamomile, ampicillin, lipopolysaccharide, male reproductive system, fertility, mice

Introduction

A wide range of bacteria in varying degrees are involved in male infertility [1, 2].It can also indirectly cause infection, testis damage, inflammation, and immune system stimulation against self-antigens along with leukocytospermia, all of which can lead to male infertility [3]. In LPS-induced sperm leukocyte inflammation, leukocyte expression pattern diagnosis receptors, member of TLRs family, are involved. The pattern of expression and diagnosis of this receptor on various immune cells is highly protected. TLRs play a key role in the expression of innate and inflammatory immune system [4]. TLR2 and TLR4 are also expressed in sperm [5]. Their activity leads to the expression of cytokines and chemokine genes, activation of the NFκβ factor, TNFα necrosis tumor factor, which plays an important role in cell apoptosis and reducing sperm motility [6]. In this regard, Escherichia coli are one of the most important symptomatic and asymptomatic infections of the genital tract and can change parameters such as motility and metabolism. It has also been shown that the presence of Escherichia coli leads to the death of 80% of sperms in conditions out of the body [7].

One of the important aspects in this regard is the emergence of microbial-resistant strains. Given the measures taken so far to produce broad-spectrum antimicrobial drugs, the issue of the emergence and prevalence of microbial resistances, especially the gram-negative bacteria resistances, is considered as major barrier in definitive treatment

of infectious diseases**.** Among these bacteria, the bacteria producing broad-spectrum beta-lactamase due to the hydrolysis of many beta-lactam group antibiotics, such as penicillin, ampicillin, cephalosporin and aztreonam, have caused many problems in treating the infectious diseases caused by these bacteria [8]. The phenomenon of multiple microbial resistances to antibiotics has been reported among isolates creating extended spectrum β-lactamase (ESBL), led to reduced effect of these drugs on bacteria [9]. Escherichia coli are one of the common species ESBL enzymes are key. Studies conducted around the world have shown resistance to various antibiotics on Escherichia coli isolated from patients' samples [10]. Nowadays, the use of herbs and their compounds has been proposed as a substitute for common antibiotics**.** Results of many studies have showed that extracts of some plants can inhibit the growth of microorganisms, and medicinal plants as antimicrobial agents have found new applications including in medicine, food industry, etc. [11]. Antimicrobial compounds of medicinal herbs are one of the valuable resources in medicine. The identification of more of these plants and the purification of their compounds can be useful in the treatment of diseases. Not only do they contribute to the treatment of infectious diseases, but also reduce the many side effects often associated with the use of antibiotics [12].

Based on ancient Iranian medicine, chamomile is hot and dry plant, and it is used as a sexual drive stimulator. Given the long history of traditional medicine and the use of medicinal plants in the treatment of diseases, the lack of attention to medicinal plants in the present age, the serious side effects of chemical drugs, the high cost of chemical drugs compared to herbal medicines, the spread of antibiotic resistance as a serious global threat, the presence of bacterial agents (especially gram-negative bacteria) as the main causes of male infertility, the beneficial effects of the herbal medicines should be proven in clinical trials from the research-scientific perspective.

Regarding the medicinal effects of this plant, the aim of this study was to investigate the effect of chamomile extract in comparison with ampicillin on reproductive histology and fertility rates in male mice treated with *E. coli* lipopolysaccharide.

Materials and Methods

- **Experimental animals**

Sixty-five mature mice in the weight range of 25 to 30g were used. Animals were kept in a temperature and humidity Controlled room. Samples had free access to food and water, 12:12 hour's light-darkness cycle for 10 days. Cage floor were covered with sawdust which were replaced every 2 days. In the present study, ethical principles were observed in accordance with the rules of support and maintenance of laboratory animals and statements of animal researches committee, Vale do Paraiba University.

- **Grouping experimental samples**

The classification of groups is as follows:

1) Control group
2) LPS *E. coli* group
3) LPS + ampicillin group
4) LPS + chamomile extract with dose of 50 mg/kg
5)LPS + Chamomile extract with dose of 100 mg/kg
6) LPS + Chamomile extract with dose of 200 mg/kg

Grouping the male mice at the end of experiment for mating and fertility. One week before the end of the 30-day injection period (to adapt the male mice to the new environment, where they are supposed to mate), four male mice were selected from each of the 6 experimental groups randomly (a total of 24 male mice) and they placed individually in 24 separate cages. It should be noted that the ambient temperature, access to water and food, and especially their injections, were performed based on previous procedure**.**

- **Preparation of ampicillin antibiotic**

Ten vials of ampicillin (ampixil; 1 mg) were prepared. The vials were kept in a dry and cold refrigerated standard temperature of laboratory.

- **Preparation of bacterial Lipopolysaccharide (LPS *E. coli*)**

The commercial lipopolysaccharide *E.coli* 10 mg was prepared from Sigma Code Company (L2880) by Yekta Gostar Vision Company. Nine mg of LPS lyophilized vial were measured in sterile conditions by digital scales. Then, 150 cc of the injectable serum was added and regenerated. Then, the obtained lipopolysaccharide solution was kept at a temperature of 2-8 $^{\circ C}$ in the laboratory refrigerator, to be used in the next steps of the experiment.

- **The method of injections**

Two weeks before implementing the main steps of the experiment (during a period specified for male mice adaptation to the environment) on the 5 male mice, considered for LPS test, intravenous injection of 1 mg/kg LPS was performed in two replicates with interval of five days to ensure the survival of mice in this dose**.**

- **Main *steps of injections***

The duration of the experiment lasted 30 days, which is as follows**:**

1- The control group received only daily water and food.
2- Intravenous injection of 1 mg/kg LPS every 5 days in 5 treatment groups LPS, ampicillin, chamomile with dose of 50, 100 and 200.
3- Intravenous injection of the hydro alcoholic extracts of chamomile at dose of 50 mg/kg, 100 mg/kg, and 200 mg/kg to three groups of chamomile every two days.
4- Daily intravenous injection of ampicillin at dose of 100 mg/kg to the ampicillin treatment group.

- **Mating and examining the number of embryo**

At the end of the 30th day of injection (one week after the transfer of male mice to a separate cage), 24 female mice were transmitted to male mice cage for mating. (Monogamy system: in this system, a pair of male and female mice is kept in a cage). After 7-10 days, male mice were separated from the female mice by identifying the signs and ensuring the fertility. The fertility period of female mice lasted 19-21 days, and the number of embryos was counted in each group at that end of the fertility period**.**

- **Steps of performing histological experiments and examining histological factors**

After fixation, tissue passage and preparation of tissue slices with a thickness of 5 μm and staining with hematoxylin-eosin, tissue slides were prepared serially. Then, the number of stem cells, primary spermatocyte with magnification of ×40 and diameter of the diameter of seminiferous tubules with magnification of ×10 were counted and measured in fields of view, selected randomly from the slides. Accordingly, for each group, 4 slides, and 5 seminiferous tubules from each slide were selected randomly and three histological factors were evaluated**.**

- **Statistical analysis**

In this study, to compare the mean of the data obtained from the experiment result in order to examine the existence or absence of significant differences between the groups, one-way ANOVA analysis, Tukey (HSD) test, and SPSS software were used. $p<0.05$ was considered as a significance level. The Minimum Standards of Reporting Checklist contains details of the experimental design, and statistics, and resources used in this study.

Results and Discussion

The results of examining the testis histological sections under the optical microscope are as follows**:**

- **Examining the counting the number of stem cell**

After examining and counting the number of testis stem cells using histological sections, the mean number of stem cells in the group received LPS alone showed significant reduction compared to that of control group ($p\leq0.05$). Significant difference was found between ampicillin and chamomile groups in three doses of 50-100-200 mg/kg and control group ($p<0.05$) (Figure 1).

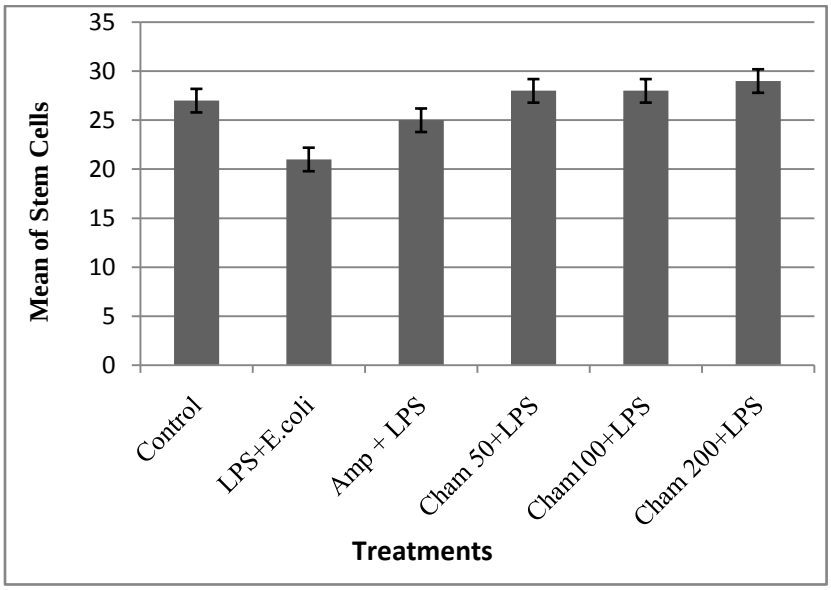

Figure 1: Stem cell changes in testis tissue in control and treatment samples

- **Examining the number of primary spermatocyte**

There was significant difference between the mean number of primary spermatocyte cells in the treatment groups with LPS, ampicillin and chamomile in the three doses of 50, 100 and 200 mg/kg and that of control group ($p<0.05$) (Figure 2).

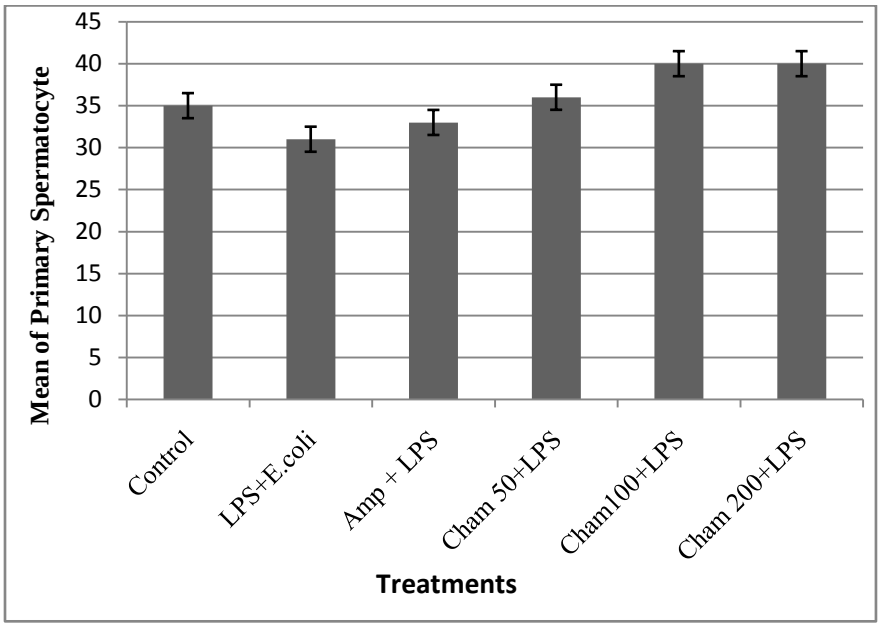

Figure 2: Changes in primary spermatocyte cells in testis tissue in control and treatment samples

There was no significant difference between the histological characteristic in the treatment groups with LPS, ampicillin and chamomile in the three doses of 50, 100 and 200 mg/kg and that of control group ($p <0.05$)

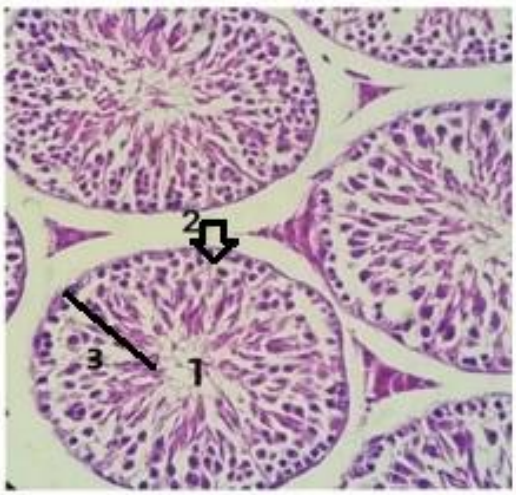

Figure 3: Cross section of seminiferous tubules in the control group with magnification 40x10
1= Lumen of seminiferous tubule; 2= Seminiferous tubul;
3= Varying Stages Of Sperm Development

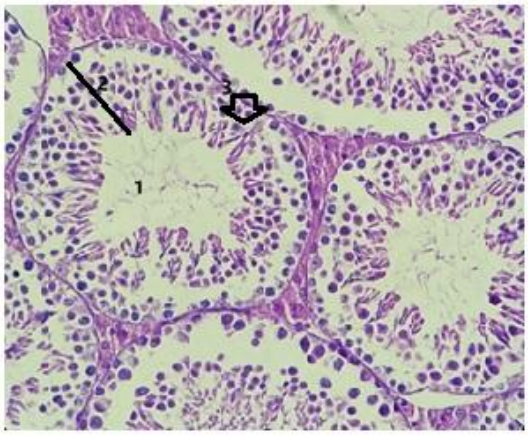

Figure 4: Cross section of seminiferous tubules in the experimental group LPS with a magnification of 40x10
1= Lumen of seminiferous tubule; 2= Varying Stages Of Sperm Development;
3= Seminiferous tubule

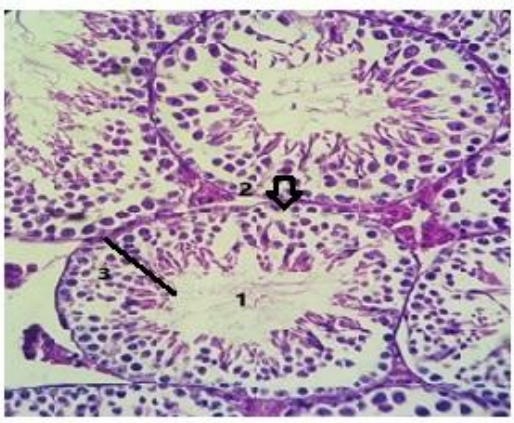

Figure 5: Cross section of seminiferous tubules in the experimental ampicillin+LPS with a magnification of 40x10
1= Lumen of seminiferous tubule; 2= Seminiferous tubule
3= Varying Stages of Sperm Development

- **Examining the diameter of the seminiferous tubules**

After examining and measuring the diameter of seminiferous tubules by Eye Piece Micrometer in histological sections, significant difference was found between the mean diameter of the seminiferous tubules of LPS, ampicillin and chamomile groups in three doses of 50, 100 and 200 mg/kg and that of control group ($p>0.05$) (Figure6).

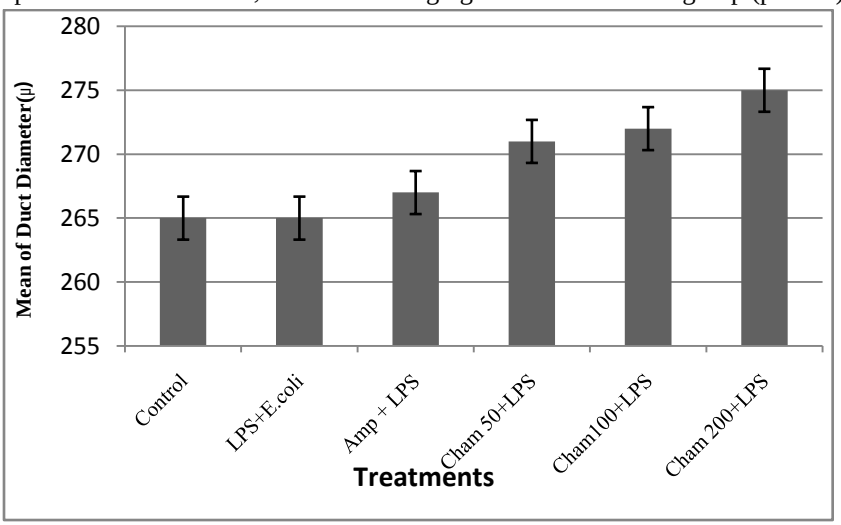

Figure 6: Changes in diameter of seminiferous tubules in testis tissue of control and treatment samples

- **Examining the number of embryos**

After fertilization and counting the number of embryos in each group, and comparing the mean number of embryos of LPS, ampicillin and chamomile groups in three doses of 50 and 100 mg/kg with mean of control group did not show any significant difference ($p <0.05$) (Figure 7).

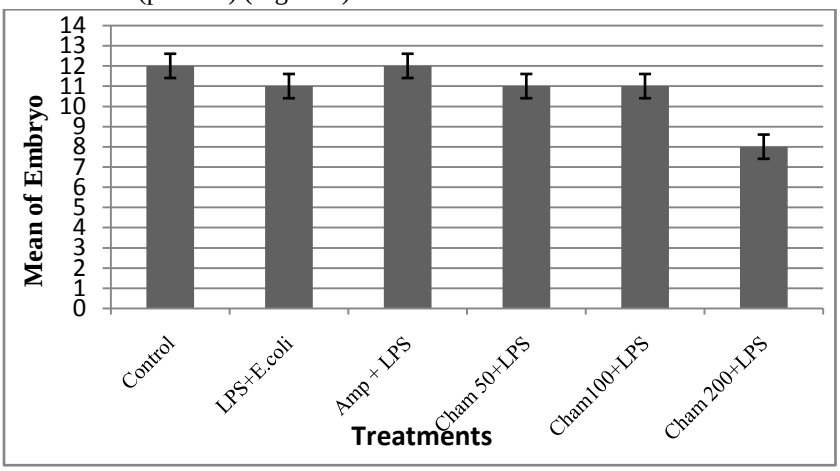

Figure 7: Changes in the number of embryos in control and treatment samples

The results of the present study showed a significant reduction in the mean number of stem cells in the LPS group compared to that in the control group and the mean number of primary spermatocyte cells in the treatment groups of LPS, ampicillin and chamomile in three doses of 50, 100 and 200 mg/kg show significant difference compared to that of control group. In addition, the mean diameter of the seminiferous tubules of groups LPS, ampicillin and chamomile in three doses of 50, 100 and 200 mg/kg was significantly different from that of the control group. It could be stated that the infection caused by 1 mg/kg dose of LPS reduced stem cells and number of primary spermatocytes, and this reduction in the number of stem cells in other groups was compensated by antibiotics ampicillin and chamomile extract in doses of 50, 100 and 200 mg/kg and approached to the number of stem cells in

the control group and it did lead to significant change probably due to the lack of involving the primary spermatocytes and the diameter of the seminiferous tubules with infection, so the antibiotic effect of ampicillin and the antibacterial properties of the chamomile extract did not affect them.

Other finding of this study suggests that the mean number of embryos in the treatment groups of LPS, ampicillin and chamomile in the three doses of 50 and 100 mg/kg did not differ significantly with that of the control group. Thus, based on these results, the infection caused by a dose of 1 mg/kg LPS might not have an effect on the number of embryos, but due to non-involvement of sperm and fertility with the infection, the antibiotic effect of ampicillin and antibacterial properties of the chamomile extract were not influential. In LPS-induced sperm leukocyte inflammation, leukocytes expression pattern diagnosis receptors, members of Toll-like receptors (TLRs), are involved**.** The expression pattern and diagnosis of this receptor is strongly protected on various immune cells. TLR plays a key role in the expression of innate and inflammatory immune system [4]. Studies have shown that an increase in the number of leukocytes in semen (leukocytospermia) plays an important role in male infertility [13]. Leukocytes are one of the major sources of reactive oxygen species in semen. It has not been revealed well how these cells enter the seminiferous tubules, but studies have shown that infection removes the strong connections between the sertoli cells or their resistance. As a result, leukocytes invade into seminiferous tubules[14].

Thus, it could be stated that the infections caused by LPS 1 mg/kg led into activation of the Toll-like receptors, and then, the strong connections between the sertoli cells were removed or reduced, and then, the leukocytes invaded into seminiferous tubules, led to production of more ROS by leukocytes (oxidative stress) [15]. As a result, it affected the self-renewal characteristic of stem cells, leading to a reduction in this type of cells in testis tissue. However, it did not have an effect on differentiation potential of these cells to other testis tissue cells, including primary spermatocyte**.**Given the studies conducted, the compounds of chamomile extract have anti-inflammatory, anti-bacterial and antioxidant activity [16]. The chamomile plant is also rich in flavonoids, which have effective antioxidants in neutralizing oxidative radicals [17]. Another study reported that chamomile has been used for treating inflammatory diseases in traditional medicine since old days [18]. Chamomile essential oil (CEO) treatment significantly prevented the damage caused by daunorubicin (DAU) in mice, led to reduction in level of sister-chromatid exchange (SCE) inspermatogonia. This information also showed the antioxidant capacity of the essential oil [19]. Previous studies have also examined the antimicrobial effects of ethanol extracts of 45 and 90% of chamomile aerial parts on a number of pathogenic fungi and bacteria.

The results of these studies showed that both of the tested extracts had a significant effect on the bacteria and fungi studied, but the second extract of ethanol extract 90 $^{\circ C}$ had significantly stronger effect than the first extract of ethanol extract 45 $^{\circ C}$[20]. With regard to mechanism of action of essential oils on the death of pathogenic bacteria, it has been stated that one of the important characteristics of these substances and their compounds is hydrophobicity characteristic, which causes distribution of bacteria in lipid parts of cell mitochondrial walls, leading to change in destruction in their building and more permeability of them [21]. Then, major parts of ions and other vital contents of the cell leak out, leading bacterial death [22]. Several studies have confirmed the antimicrobial role of chamomile extract on Escherichia coli in In-vitro condition, which is consistent with the results of this study, conducted in In-vivo conditions. Thus, the results of this study are confirmed by previous studies. Hence, given the results of previous studies and the results of this study, it could be stated that chamomile extract induced its therapeutic effect due to its antibacterial property, as ampicillin antibiotic. Penetrating into the membrane of the bacterial cell, chamomile extract also leads to swollen membrane, and thus, cell death and loss of infection. As a result, the reduction in the number of testis stem cells in the LPS group was compensated compared to control group. However, due to non- involvement of primary spermatocytes with bacterial infection, the antimicrobial activity of the ampicillin antibiotic and chamomile extract did not affect the number of primary spermatocytes.

Moreover, infection with LPS 1 mg/kg did not affect the diameter of the seminiferous tubules and the number of embryos. Therefore, it could be stated that due to non- involvement of seminiferous tubules and sperm with microbial infection and the resulting infection, the antimicrobial activity of the ampicillin antibiotic and chamomile extract did not affect the diameter of the seminiferous tubules and the number of embryos**.** It seems that essential oil

of German chamomile to have significant impact on negative-gram bacterium of *E. coli* and significant anti-microbial effect compared toampicillin antibiotic.

Conclusion

According to the results, the use of chamomile extract in a dose-dependent manner as an antibacterial, anti-inflammatory and antioxidant agent in improving the infection compared to antibiotic ampicillin can reduce the number of spermatogenic cells in testis.

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